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Original research

Sublethal effects of chlorpyriphos on some hematological parameters in freshwater fish *Anabas testudineus*

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Abstract: Hematological parameters are often used to assess the health status and as stress indicators in fish. The aim of this study was to assess the sublethal effects of chlorpyriphos, an organophosphate insecticide, on some hematological parameters in freshwater fish *Anabas testudineus.* The effect was assessed based on the comparison results of control group and experimental groups exposed to sublethal concentrations (0.125, 0.250, 0.375 mg L^{-1}) of chlorpyrifos at 7th, 14th and 21st days. It was observed that with the increase of chlorpyrifos concentrations and days, red blood cell counts, hemoglobin levels, hematocrit levels and thrombocyte counts decreased. White blood cell counts increased at 7th day of exposure but decreased with the increase of chlorpyrifos concentrations at 14th and 21th days. The study showed that the chlorpyrifos significantly impacted the health status of the fish and hematological parameters may be useful as a diagnostic test for chlorpyrifos exposure in *A. testudineus*.

Keywords: chlorpyrifos, organophosphate insecticide, *Anabas testudineus*, hematological parameters

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Introduction

Organophosphates are some of the most widely used pesticides in the world. They are used in agriculture, homes, gardens, and veterinary practices. In general, they are not persistent in the environment as they break down quickly. Because of their relatively fast rate of degradation, they have been a suitable replacement for the more persistent organochlorine (Frederick, 2005).

Chlorpyrifos is a broad-spectrum organophosphate insecticide used for the control of mosquitos, flies, various crop pests in soil and on foliage, household pests and aquatic larvae. It may be used in some countries as an aquatic larvicide for the control of mosquito larvae (WHO, 2003). Chlorpyrifos reaches aquatic bodies through direct application or agricultural runoff from treated areas. Direct application of chlorpyrifos or agricultural runoff from treated areas can result in contamination of chlorpyrifos up to 4.3 μg L^{-1} in streams and lakes (Thomas and Nicholson, 1989; Richards and Baker, 1993).

Chlorpyrifos is highly toxic to freshwater fish. The values range between 26 µg L^{-1} and 33 mg L^{-1} at different fish species (Paracampo et al., 2015). Fishes are sensitive to exposure of low chlorpyrifos concentrations, and this has been shown in several studies and it was reported that chlorpyrifos induced behavioral (Levin et al., 2004), morphological (Sledge et al., 2011), biochemical (Kadam and Patil 2016) and histopathological changes in the fish (Velmurugan et al., 2015). However, very little information is available regarding the hematological effects of chlorpyrifos on different fish species (Giron-Perez et al., 2006; Ramesh and Saravanan, 2008; Malla et al., 2009).

The hematological parameters of fish provide a suitable tool, that can be a good indicator of stress and health disturbances induced by biotic and abiotic agents of the environment. In addition, they have proven useful for the detection and diagnosis of metabolic alterations and disease processes due to pesticides (Luskova, 1997).

Anabas testudineus is a fish which can tolerate extreme conditions. It is widely seen in canals, lakes, ponds, swamps and inland water bodies of India, hence selected as biological indicators of ecotoxicological studies. However, no information is available regarding the hematological effects of chlorpyrifos on *A. testudineus*. Such studies would provide much needed information required for an appropriate risk assessment of chlorpyrifos. Therefore, in the present investigation, it was decided to determine the haematological parameters as biomarker in fish *A. testudineus* exposed to sublethal concentrations of chlorpyrifos.

Materials and methods

Animals and experimental design

Fish were collected from a freshwater source in the Puzhal Lake, Redhills, Chennai, Tamil Nadu, India. The specimens were transported to the laboratory in appropriately aerated plastic bags. Fish, *A. testudineus* with weight 72 ± 5 g and length 16 ± 1 cm mean ± SD was utilized as the model organisms in this study. The fish were acclimated to the laboratory conditions for at least 20 days prior to the experiment in a glass aquarium. The fish were fed daily with commercially balanced fish food sticks.

The fish were divided into four groups and placed in separate glass aquaria. Present study was performed in three replicates in order to ensure the reproducibility of the results. Ten fish were used for each group per replicate. Group I was maintained in pesticide-free water to serve as control. Groups II, III and IV were exposed to sublethal concentration of chlorpyrifos. The sublethal concentrations of chlorpyrifos tested were 0.125, 0.250 and 0.375 mg L^{-1} (5%, 10% and 15% respectively of value). The LC_{50} for chlorpyrifos is 2.5 mg L^{-1} for *A. testudineus* (Velmurugan et al., 2015).

Sampling and analysis

At 7th, 14th and 21st days of treatment, both the experimental and control fish were anesthetized using tricaine methanesulphonate. Anesthetized fish were used for collecting blood samples. Blood was obtained by severance of caudal peduncle and collected in Eppendorf tubes containing EDTA anticoagulant and mixed immediately (Mgbenka et al., 2003). These exposed and control anticoagulant blood samples were used for haematological parameters. The hematological parameters analysed were RBC, WBC, Hgb, Hct and thrombocytes. Due to less amount of blood present in the fish for accuracy fully automated cell counter - sysmax - 21 from Japan was used for analyzing hematological parameters (Maceda-Veiga et al., 2010).

Statistical analysis

One way ANOVA was used to evaluate measurements in this study. The Tukey HSD, Dunnet test was used for multiple comparisons. Normal distributions were evaluated using the Kolmogorov-Smirnow test and homogeneity was evaluated using Levene's test. All data were analyzed using the statistical package SPSS version 15.0 for Windows. The significance of test results was ascertained at $P < 0.05$.

Results

Red blood cell (RBC)

In control, the RBC count was recorded as 4.08 ± 0.34 10^6 μL^{-1} at 7th day. The RBC counts were observed as 3.61 ± 0.35 10^6 μL^{-1} at 0.125 mg L^{-1}, as 3.32 ± 0.24 10^6 μL^{-1} at 0.250 mg L^{-1} and as 3.15 ± 0.28 10^6 μL^{-1} at 0.375 mg L^{-1}. In control, this count was recorded as 4.11 ± 0.27 10^6 μL^{-1} at 14th day. In control, the RBC count was recorded as 4.19 ± 0.27 10^6 μL^{-1} at 21 st day. The RBC counts were in decreasing trend with increase in the concentrations of chlorpyrifos at 7th, 14th and 21st days. The overall decline was observed throughout the exposure period. The

maximum decrease was found at concentration 0.375 mg L^{-1} as 0.98 ± 0.20 10^6 μL^{-1} at 21st day. In the present study, significant changes of RBC were presented in Table and Figure 1.

Table Hematological values of *Anabas testudineus* exposed to sublethal concentrations of chlorpyrifos.

Hematological parameters	Chlorpyrifos concentrations (mg L^{-1})	7th day	14th day	2st day
RBC (10^6 μL^{-1})	Control	4.08 ± 0.34^{ax}	4.11 ± 0.27^{ax}	4.19 ± 0.27^{ax}
	0.125	3.61 ± 0.35^{bx}	3.24 ± 0.30^{by}	2.73 ± 0.20^{bz}
	0.250	3.32 ± 0.24^{bcx}	3.02 ± 0.23^{bcy}	2.48 ± 0.24^{bz}
	0.375	3.15 ± 0.28^{cx}	2.87 ± 0.19^{cy}	0.98 ± 0.20^{cz}
Hgb (g dL^{-1})	Control	16.45 ± 0.93^{ax}	16.81 ± 1.08^{ax}	17.05 ± 1.01^{ax}
	0.125	15.26 ± 1.15^{abx}	11.56 ± 1.02^{by}	10.98 ± 1.13^{by}
	0.250	14.86 ± 0.98^{bx}	10.90 ± 0.83^{by}	8.48 ± 0.78^{cz}
	0.375	11.37 ± 0.91^{cx}	6.15 ± 0.93^{cy}	4.27 ± 0.75^{dz}
Hct (%)	Control	49.67 ± 1.53^{ax}	50.50 ± 1.60^{ax}	51.00 ± 1.61^{ax}
	0.125	48.72 ± 1.07^{abx}	36.13 ± 1.64^{by}	32.44 ± 1.38^{bz}
	0.250	47.59 ± 1.60^{bx}	34.40 ± 1.75^{by}	31.63 ± 2.32^{bz}
	0.375	38.47 ± 1.36^{cx}	28.64 ± 0.98^{cy}	12.82 ± 1.62^{cz}
WBC (10^3 μL^{-1})	Control	195.00 ± 12.67^{ax}	194.10 ± 9.57^{ax}	198.40 ± 9.36^{ax}
	0.125	206.20 ± 9.46^{abx}	159.20 ± 13.69^{by}	135.40 ± 11.65^{bz}
	0.250	211.10 ± 10.90^{bx}	140.50 ± 13.95^{cy}	117.30 ± 11.74^{cz}
	0.375	227.30 ± 9.14^{cx}	128.10 ± 13.81^{cy}	95.60 ± 14.87^{dz}
Thrombocyte (10^3 μL^{-1})	Control	41.70 ± 5.44^{ax}	43.30 ± 5.12^{ax}	42.80 ± 4.94^{ax}
	0.125	23.30 ± 4.72^{bx}	21.50 ± 5.58^{bx}	15.00 ± 4.22^{by}
	0.250	17.10 ± 3.96^{cx}	14.40 ± 4.14^{cxy}	12.80 ± 3.08^{bcy}
	0.375	16.50 ± 4.67^{cx}	11.70 ± 3.06^{cy}	9.60 ± 2.88^{cy}

Values are expressed as mean ± SD (N = 10). Letters "a," "b," "c"and "d" and indicate differences between groups at the same time, and letters "x", "y" and "z"and indicate differences between times for the same group. $P < 0.05$.

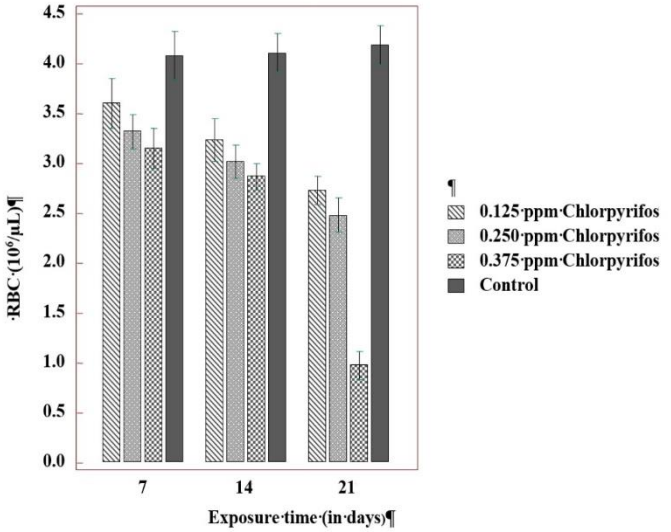

Figure 1. Red blood cell counts in *Anabas testudineus* exposed to sublethal concentrations of Chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

Haemoglobin (Hgb)

In control, the Hgb level was recorded to be 16.45 ± 0.93 g dL^{-1} at 7th day. These levels were observed as 15.26 ± 1.15 g dL^{-1} at 0.125 mg L^{-1}, as 14.86 ± 0.98 g dL^{-1} at 0.250 mg L^{-1} and as 11.37 ± 0.91 g dL^{-1} at 0.375 mg L^{-1}. In control, the level was obtained to be 16.81 ± 1.08 g dL^{-1} at 14th day. In control, the Hgb level was recorded to be 17.05 ± 1.01 g dL^{-1} at 21st day. The Hgb levels were observed as 10.98 ± 1.13 g dL^{-1} at 0.125, as 8.48 ± 0.78 g dL^{-1} at 0.250 mg L^{-1} and as 4.27 ± 0.75 g dL^{-1} at 0.375 mg L^{-1}. The Hgb levels were in decreasing trend with an increase in the concentrations of chlorpyrifos at 7th, 14th and 21st days. The overall decline was observed throughout the exposure period. The maximum decrease was found at concentration 0.375 mg L^{-1} as 4.27 ± 0.75 g dL^{-1} at 21st day. In the present study, significant changes of Hgb levels were presented in Table and Figure 2.

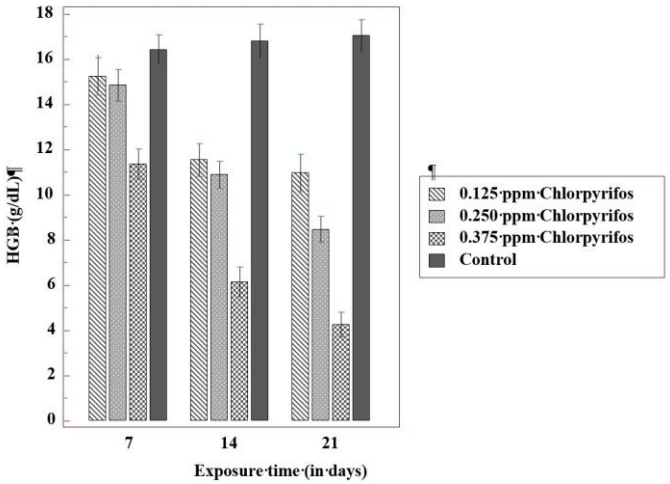

Figure 2. Hemoglobin levels in *Anabas testudineus* exposed to sublethal concentrations of Chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

Hematocrit (Hct)

In control, the Hct level was recorded to be 49.67 ± 1.53 % at 7th day. These levels were observed as 48.72 ± 1.07 % at 0.125 mg L^{-1}, as 47.59 ± 1.60 % at 0.250 mg L^{-1} and as 38.47 ± 1.36 % at 0.375 mg L^{-1}. In control, the level was 50.50 ± 1.60 % at 14th day. These levels were found as 32.44 ± 1.38 % at 0.125 mg L^{-1}, as 31.63 ± 2.32% at 0.250 mg L^{-1} and as 12.82 ± 1.62 % at 0.375 mg L^{-1}. The Hct levels were in decreasing trend with an increase in the concentration of chlorpyrifos at 7th, 14th and 21st days. The overall decline was observed throughout the exposure period. The maximum decrease was found at a concentration of 0.375 mg L^{-1} as 12.82 ± 1.62 % at 21st day. In the present study, significant changes in Hct levels were displayed in Table and Figure 3.

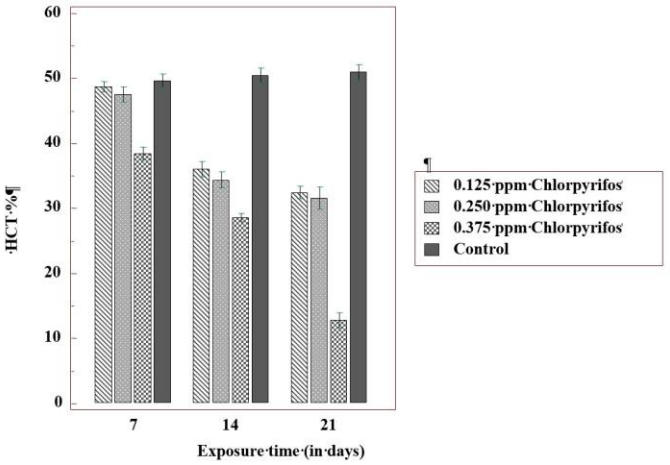

Figure 3. Hematocrit levels in *Anabas testudineus* exposed to sublethal concentrations of Chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

White blood cell (WBC)

In control, the WBC count was recorded to be 195.00 ± 12.67 10^3 μL^{-1} at 7th day. These counts were observed as 206.20 ± 9.46 10^3 μL^{-1} at 0.125 mg L^{-1}, as 211.10 ± 10.90 10^3 μL^{-1} at 0.250 mg L^{-1} and as 227.30 ± 9.14 10^3 μL^{-1} at 0.375 mg L^{-1}. The counts elevated with an increase in the concentrations of chlorpyrifos at 7th day. In control, the WBC count was recorded to be 194.10 ± 9.57 10^3 μL^{-1} at 14th day. The WBC counts when exposed to 0.125, 0.250 and 0.375 mg L^{-1} were obtained as 159.20 ± 13.69 10^3 μL^{-1}, 140.50 ± 13.95 10^3 μL^{-1} and 128.10 ± 13.81 10^3 μL^{-1}, respectively. They declined with an increase in the concentrations of chlorpyrifos at 14th day. In control, the count was recorded to be 198.40 ± 9.36 10^3 μL^{-1} at 21st day. They were observed as 135.40 ± 11.65 10^3 μL^{-1} at 0.125 mg L^{-1}, as 117.30 ± 11.74 10^3 μL^{-1} at 0.250 mg L^{-1}, and as 95.60 ± 14.87 10^3 μL^{-1} at 0.375 mg L^{-1}. The WBC counts were in decreasing trend with an increase in the concentrations of chlorpyrifos at 21st day. The WBC counts showed changes in *A. testudineus* exposed to sublethal concentrations of chlorpyrifos at 7th, 14th and 21st days. The increasing counts of WBC were observed at 7th day and the elevation was found to be dose dependant. The decreasing counts of WBC were observed at 14th and 21st days. The maximum decline was found as 95.60 ± 14.87 10^3 μL^{-1} at 0.375 mg L^{-1} at 21th day. In the present study, significant changes of WBC were presented in Table and Figure 4.

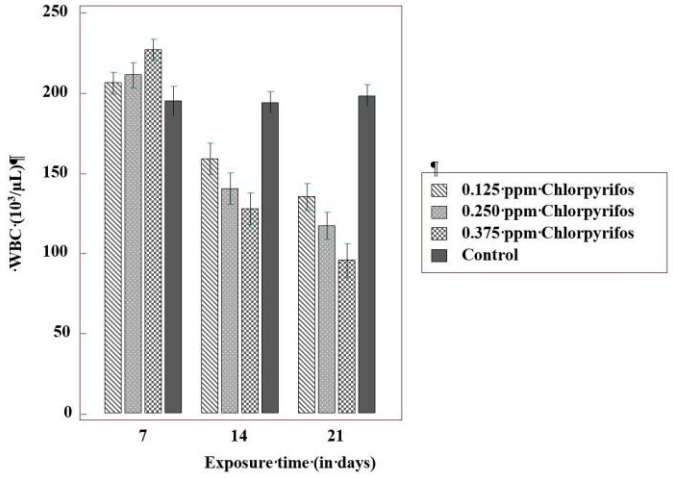

Figure 4. White blood cell counts in *Anabas testudineus* exposed to sublethal concentrations of Chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

Thrombocyte

In control, the thrombocyte count was recorded to be 41.70 ± 5.44 10^4 μL^{-1} at 7th day. The thrombocyte counts were 23.30 ± 4.72 10^4 μL^{-1} at 0.125 mg L^{-1}, 17.10 ± 3.96 10^4 μL^{-1} at 0.250 mg L^{-1} and 16.50 ± 4.67 10^4 μL^{-1} at 0.375 mg L^{-1}. In control, the count was 43.30 ± 5.12 10^4 μL^{-1} at 14th day. These counts were 21.50 ± 5.58 10^4 μL^{-1} at 0.125 mg L^{-1}, 14.40 ± 4.1410^4 m μL^{-1} at 0.250 mg L^{-1} and 11.70 ± 3.06 10^4 μL^{-1} at 0.375 mg L^{-1}. In control, the count was 42.80 ± 4.94 10^4 μL^{-1} at 21st day. They were 15.00 ± 4.22 10^4 μL^{-1} at 0.125 mg L^{-1}, 12.80 ± 3.08 10^4 μL^{-1} at 0.250 mg L^{-1} and 9.60 ± 2.88 10^4 μL^{-1} at 0.375 mg L^{-1}. The thrombocyte counts decreased with an increase in the concentration of chlorpyrifos at 7th, 14th and 21st days. The maximum decrease was found at a concentration of 0.375 mg L^{-1} as 9.60 ± 2.88 10^4 μL^{-1} at 21st day. In the present study, significant changes of thrombocyte counts were introduced in Table and Figure 5.

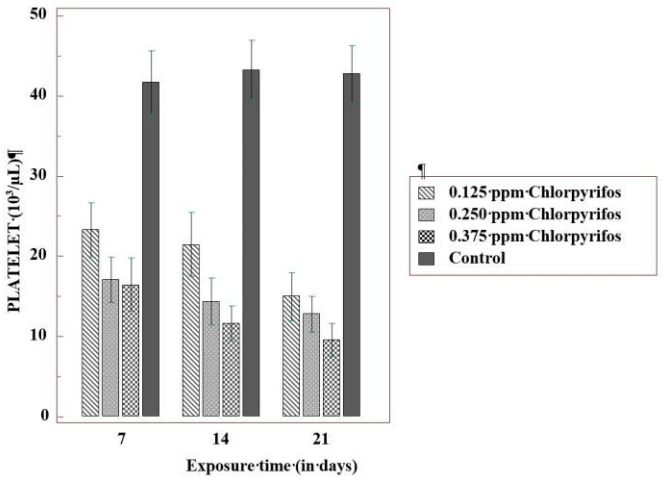

Figure 5. Platelet counts in *Anabas testudineus* exposed to sublethal concentrations of Chlorpyrifos for 7, 14 and 21 days. Data are expressed as mean± SD (N = 10). $p<0.05$.

Discussion

Haematological parameters have been recognized as valuable tools for monitoring fish health (Satheeshkumar et al., 2011).

In the present study, significant alterations in the haematological parameter of *A. testudineus* exposed to sublethal concentrations of chlorpyrifos were observed. The results obtained in this study showed decreases in RBC counts, Hgb and Hct levels. These results are consistent with the findings of other studies investigating hematological response of different fish species exposed to other organophosphate pesticides. Similar reductions in RBC counts and Hgb levels had been also reported after exposure to diazinon in *Cyprinus carpio, Silurus glanis*, *Clarias gariepinus* and *Cirrhinus mrigala* (Svobodam et al., 2001; Koprucu et al., 2006; Adedeji et al., 2009; Haider and Rauf, 2014) methyl parathion in *Mystus keletius* (Sampath et al., 2003) monocrotophos *in Clarias gariepinus* (Yagi and Auta, 2007), *Cyprinus carpio* exposed to chlorpyrifos (Ramesh and Saravanan, 2008). But, Giron-Perez et al. (2006) showed that chlorpyrifos did not affect the Hgb levels at any of the concentrations, compared with control group in *Oreochromis niloticus*.

The significant reduction in RBC counts may be due to disruptive action of the chlorpyrifos on the erythropoietic tissue. The significant decrease in the Hgb levels may also be due to either an increase in the rate at which the Hgb is destroyed or to a decrease in the rate of Hgb synthesis (Reddy and Bashamohideen, 1989).

Increased destruction of RBC can lead to decreased hemoglobin. The decrease in Hct in fish exposed to chlorpyrifos was due to decreased RBC count.

In the present study, WBC count increased initially at 7th day of exposure. At 14th and 21st days of exposure, WBC counts decreased. The increases in WBC counts (leucocytosis) were reported in *Clarias gariepinus* exposed to monocrotophos (Yagi and Auta, 2007), *Cyprinus carpio* exposed to acute toxicity of chlorpyrifos (Ramesh and Saravanan, 2008). However, the decrases in WBC counts (leucopenia) were also described in the *Silurus glanis, C. carpio* and *C. gariepinus* exposed to diazinon (Koprucu et al., 2006; Banaee et al., 2008; Adedeji et al., 2009) in *Oreochromis mossambicus* exposed to phosalone (Jaffar and Rani, 2009). The total WBC count of *Cirrhinus mrigala* exposed to diazinon showed a delayed decrease. The WBC counts of controlled and exposed fish were not significantly different from each other after 10 days of exposure to diazinon, but after 20 and 30 days of exposure, a significant decrease in WBC count of both exposed fish groups was observed when compared to controlled fish group (Haider and Rauf, 2014).

The increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to sublethal

concentrations of pesticide (Joshi et al., 2002). Reduced numbers of leucocytes in exposed fish can result in reduced disease resistance (Kaattari and Piganelli, 1996).

In this study was significant reduction in thrombocyte counts with the exposure of the fish to chlorpyrifos. But, Adedeji et al. (2009) reported a significant increase in thombocyte count in *Clarias gariepinus* exposed to diazinon.

The decrease of this parameter can be related with trapping of thrombocytes in the spleen, decreased thrombocyte production or increased destruction of thrombocytes.

The present study showed the toxicity of chlorpyrifos on the haematology of *A. testudineus* and the significance of hematological parameters in assessing the chlorpyrifos hazards to *A. testudineus*. The sub-lethal exposure to clorpyrifos for 21 days resulted significant hematological changes. The hematological disturbances which could lead to impairment of fish ability to combat diseases reduce its chances for survival for growth. This study clearly indicates that the presence of chlorpyrifos in fresh water reservoirs, even in small concentration, could cause deleterious effects on fish physiology and may potentially disturb their survivability in the natural environment. Therefore, controlling measures should be taken to prevent the possible contamination of the aquatic environment by such toxic pesticides.

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